## I. AMENDMENT TO THE SPECIFICATION

*Please amend the following paragraph starting at line 21, page 7 as follows:*

--The constructed plasmids for overexpression, containing several combinations of sec genes are shown in Table 1, plasmid maps are shown as ~~Figures 5 to 8~~ Figures 3 to 6.—

*Please amend the following paragraphs starting at line 29, page 11 as follows:*

~~--Figure 1~~

~~Schematic presentation of the chromosomal organization of the M. tuberculosis secD/secF region (a), compared with the same region in Corynebacterium glutamicum (b). In the scheme of the rescued vector (b), restriction enzymes used for plasmid rescue are in brackets and enzymes used for cloning are marked (*).~~

~~Figure 2~~

~~Comparison of the deduced amino acid sequences of SecD (a) and SecF (b) of Corynebacterium glutamicum and M. tuberculosis. Identical amino acids (*) and conservative replacements (:) are marked. The conserved regions D1-D6 and F1-F4, which are present in all known SecD and SecF Proteins are boxed. Two possible SecY interaction sites of SecF were found by analysis of the amino acid sequence with FingerPRINTscan (Attwood et al., 1999, above) and marked with black bars. The six putative transmembrane regions of SecD and SecF are indicated in grey shadings. The membrane spanning domains of all Proteins were predicted using the HMMTOP web site (Tusnády and Simon, 1998, above).~~

~~Figure 3~~ Figure 1

Amylase secretion of Corynebacterium glutamicum AMY2 overexpressing different combinations of sec genes. 1,3 X $10^6$ cells were incubated for 16 h. Activity in the supernatant was determined 5 times. 1 mU was defined as 1 nmol reducing sugar $min^{-1}$ $ml^{-1}$. A significant increase in amylase secretion could be detected if secD and secF are overexpressed (pSecDF). In Corynebacterium glutamicum AMY2/pSecEDF(pSecEDF) and Corynebacterium glutamicum AMY2/pSecYDF(pSecYDF) amylase activity is more than doubled compared with Corynebacterium glutamicum AMY2(AMY2).

~~Figure 4~~ Figure 2

Growth of different Corynebacterium glutamicum strains Corynebacterium glutamicum AMY2 (■), Corynebacterium glutamicum AMY2/pSecEDF(Δ) and Corynebacterium glutamicum AMY2/pSecYDF(•) in minimal medium with starch as sole carbon source. No growth was detectable for Corynebacterium glutamicum RES167 (◆). The slight decrease of optical density at the beginning of the curve results from degradation of insoluble parts of starch by the secreted amylase.

~~Figure 5~~ Figure 3 is a plasmid map of pSecD

~~Figure 6~~ Figure 4 is a plasmid map of pSecDF

~~Figure 7~~ Figure 5 is a plasmid map of pSecEDF

~~Figure 8~~ Figure 6 is a plasmid map of pSecYDF

~~Figure 9~~ Figure 7 is a plasmid map of pAmy

~~Figure 10~~ Figure 8 is a plasmid map of pIAmy2–

*Please amend the following paragraph starting at line 27, page 16 as follows:*

- In the Mycobacterium tuberculosis H37Rv strain, that is taxonomically closely related to Corynebacterium glutamicum, the dipeptid transporter encoding gene dciAE ~~(Fig. 1a)~~ is located downstream of secD and secF. The dciAE homolog of Corynebacterium glutamicum (ATCC 13032) was sequenced in part in a study on the rel gene (Wehmeier, L. Schäfer, A., Burkowski, A., Krämer, R., Mechold, U., Malke, H., Pühler, A. und Kalinowski, J. (1998), Microbiology 144, 1853-1862) to isolate the genes secD and secF by a chromosomal rescue technique, an 0,8 kb fragment of the dciAE gene, derived from plasmid pLW60 (Wehmeier et al., 1998) by digestion with EcoRI and BamHI was cloned into pCR2.1 and the resulting plasmid was integrated into the Corynebacterium glutamicum chromosome after electoporation via homologous recombination. Total chromosomal DNA was isolated from the resulting strain, digested with EcoRV and SspI religated and transferred to E.coliDH5αMCR. The rescue of the integrated vector with EcoRV and SspI results in a plasmid pCR2.1 carrying a 9751 bp insert including dciAE and the upstream chromosomal region ~~(Fig. 1)~~. The insert was sequenced by primer walking. By DNA sequence analysis, 8

complete and 1 partial orfs ("open reading frames"), including secD and secF could be identified on the fragment.—

*Please amend the following paragraphs starting at line 19, page 23 as follows:*

--Since SecD and SecF seems to be strong effectors on amylase secretion, the consequence of combined overexpression of secD and secF on protein export was examined. The combination secD and secF was cloned with SalI into the shuttle expression vector pEC-XK99A as described above. Corynebacterium glutamicum AMY2 was transformed with the resulting plasmids pSecDF and tested for amylase activity. As pointed out in ~~figure 3~~ figure 1, the simultaneous overexpression of secD and secF genes enhanced the amylase secretion 1,5 fold in contrast to Corynebacterium glutamicum AMY2.

To analyze the effect of parallel overexpression of auxiliary sec genes secD and secF with essential sec genes, secE and secY EcoRI were cloned into pSecDF, resulting in the plasmids pSecEDF and pSecYDF as described in example 3. SecE and SecY were cloned, because their interaction with the SecD/SecF complex is described in E. coli (Sagara, K., Matsujama, S. and Mitzushima, S., 1994, J. Bact. 176, 4111-4116). Test on amylase secretion revealed a 2,3 fold increase for Corynebacterium glutamicum AMY2/pSecEDF and gained 2,5 fold for Corynebacterium glutamicum AMY2/pSecYDF compared with Corynebacterium glutamicum AMY2 ~~(fig. 3)~~ (figure 1).--